DNA Photodamage Stimulates Melanogenesis and Other Photoprotective Responses

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Ultraviolet (UV) irradiation is a major source of environmental damage to skin. Melanin pigmentation protects against this damage by absorbing UV photons and UV-generated free radicals before they can react with DNA and other critical cellular components; and UV-induced melanogenesis or tanning is widely recognized as exposed skin's major defense against further UV damage. This article reviews extensive data suggesting DNA damage or DNA repair intermediates directly triggers tanning and other photoprotective responses. Evidence includes the observations that tanning is enhanced in cultured pigment cells by accelerating repair of UV-induced cyclobutane pyrimidine dimers or by treating the cells with UV-mimetic DNA-damaging chemicals. Moreover, small single stranded DNA fragments such as thymidine dinucleotides (pTpT), the substrate for almost all DNA photoproducts, also stimulates tanning when added to cultured pigment cells or applied topically to intact skin. In bacteria, single stranded DNA generated by DNA damage or its repair activates a protease that in turn derepresses over 20 genes whose protein products enhance DNA repair and otherwise promote cell survival, a phenomenon termed the SOS response. Interestingly, pTpT also enhances repair of UV-induced DNA damage in human cells and animal skin, at least in part by activating the tumor suppressor protein and transcription factor p53 and thus upregulating a variety of gene products involved in DNA repair and cell cycle regulation. Together, these data suggest that human cells have an evolutionarily conserved SOS-like response in which UV-induced DNA damage serves as signal to induce photoprotective responses such as tanning and increased DNA repair capacity. The responses can also be triggered in the absence of DNA damage by addition of small single-stranded DNA fragments such as pTpT. *Key words: UV response/DNA repair/photoprotection. Journal of Investigative Dermatology Symposium Proceedings 4:35–40, 1999*

Life on earth evolved in the presence of ultraviolet (UV) irradiation from terrestrial sunlight, and essentially all organisms developed photoprotective mechanisms to limit the resulting damage. In the case of human skin, two photoprotective mechanisms have been recognized. Melanin pigmentation, both constitutive (baseline) and facultative (inducible), is the major recognised form of protection against UV-induced damage (Nordlund *et al*, 1998; Pathak, 1995). Photoprotection is attributable to the fact that the melanin polymer can directly absorb UV photons, dissipating the otherwise injurious energy as heat, and can further absorb free radical species generated by the interaction of UV photons with cellular lipids and other molecules that otherwise cause oxidative damage. Scattering and reflection of UV photons by proteins in the stratum corneum is believed to be a second, albeit minor, mechanism of photoprotection, and the stratum corneum is known to thicken following UV irradiation, particularly in poorly melanized skin (McGregor and Hawk, 1999). Recent work by many groups has begun to elucidate the complex regulatory mechanisms underlying baseline and UV-induced pigmentation (Nordlund *et al*, 1998; Gilchrest *et al*, 1996), while the mechanisms responsible for stratum corneum thickening have not been studied and will not be considered further in this review.

Manuscript received December 10, 1998; revised June 4, 1999; accepted for publication June 9, 1999.

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As reviewed in recent textbooks (Nordlund *et al*, 1998; Jimbow *et al*, 1993), UV-induced melanogenesis, the so-called tanning response, is complex. Tanning has been shown to result both from direct effects of UV photons on melanocytes (Friedmann and Gilchrest, 1987) and from indirect effects mediated primarily through keratinocytes (**Fig 1**), but also at least to some degree through fibroblasts, neurons, mast cells, and possibly other cells resident in the skin.

Within the melanocyte itself, considerable evidence implicates the plasma membrane as a target for UV irradiation, with evidence for effects as diverse as transmembrane receptor clustering (Rosette and Karin, 1996), activation of phospholipase A2 with release of arachidonic acid and 12 (s) HETE (Cohen and DeLeo, 1993; Gron *et al*, 1993; Gresham *et al*, 1996), and cleavage of diacylglycerol from parent membrane lipids (DeLeo *et al*, 1984; Punnonen and Yuspa, 1992), leading to activation of protein kinase C and subsequent direct activation of tyrosinase by phosphorylation of serine residues in its cytoplasmic domain (Gordon and Gilchrest, 1989; Park *et al*, 1993, 1998; Allan *et al*, 1995; Park *et al*., 1999). This review focuses on the extensive evidence implicating UV-induced DNA damage as an initial photoreceptive event responsible for tanning, as well as for other protective cutaneous responses.

EVIDENCE THAT DNA DAMAGE STIMULATES MELANOGENESIS

The bacterial phage enzyme T4 endonuclease V is known to catalyze the initial and rate-limiting step in excision of cyclobutane pyrimidine dimers (CPD) and has no other recognized function (Grossman *et al*, 1988). This enzyme, encapsulated in liposomes to enhance its

percutaneous delivery, is termed T4N5 and has been shown to accelerate repair of CPD both in cultured cells (Ceccoli *et al*, 1989; Yarosh, 1992) and in intact skin (Yarosh *et al*, 1990). In experiments undertaken to determine whether T4N5 treatment of pigment cells *in vitro* affected the pigmentation resulting from UV irradiation, both murine S91 melanoma cells and human melanocytes exposed to solar simulating irradiation demonstrated greater melanogenesis when treated post-irradiation with T4N5 than when treated with diluent alone or with heat-inactivated enzyme (Gilchrest *et al*, 1993). These results suggested that accelerated and/or more extensive excision of CPD from UV-irradiated DNA in someway enhances tanning.

CPD, particularly thymine dimers, are the major DNA photoproducts formed after terrestrial sun exposure, accounting for approximately 75%–80% of total DNA damage, with pyrimidine (6–4) pyrimidone photoproducts accounting for most of the remainder (Setlow and Carrier, 1966). In both prokaryotic and eukaryotic cells, these photoproducts are recognized and excised by a family of DNA repair proteins, as extensively reviewed elsewhere in these Symposium proceedings. The DNA strand containing the photoproduct is excised with variable flanking sequences surrounding the affected dinucleotide. DNA polymerase then reconstitutes a normal undamaged DNA strand using the complementary strand as a template. The metabolic fate and biologic role, if any, of the excised photoproduct-containing single stranded DNA fragment is relatively unstudied. In bacteria, however, single-stranded DNA generated during the course of DNA damage and repair interacts with and activates a protease, leading ultimately to derepression of at least 20 genes involved in DNA repair, replication, and cell survival (Walker, 1984). This is termed the SOS response and serves not only to enhance survival after irradiation, but also to increase bacterial resistence to subsequent UV-induced DNA damage. Thus, if bacteria are exposed to a sublethal dose of UV irradiation, allowed to recover, and subsequently exposed to the same UV dose, the organisms more efficiently process the DNA damage and manifest enhanced survival.[1]

Because UV-induced tanning is the major recognized defense of human skin against subsequent UV damage (Nordlund *et al*, 1998; Jimbow *et al*, 1993), we postulated that tanning might be part of a mammalian SOS response and, by analogy to bacteria, that melanogenesis might be stimulated by single-stranded DNA fragments such as those excised during the course of DNA repair. Specifically, we asked whether thymidine dinucleotides, pTpT, the most common substrate for formation of UV-induced DNA photoproducts, might stimulate tanning.

THYMIDINE DINUCLEOTIDES (PTPT) AND OTHER DNA FRAGMENTS STIMULATE TANNING

Both S91 murine melanoma cells and normal human melanocytes are known to respond to UV irradiation *in vitro* with increased cellular melanin concentration (Friedmann and Gilchrest, 1987). Accordingly, paired cultures of S91 cells or human melanocytes were incubated in pTpT or diluent alone and assayed at intervals for melanin content. Within 5 d, cellular melanin concentration increased up to 7-fold in the S91 cells and up to 80% above control levels in the human melanocytes (Eller *et al*, 1994), responses very comparable with those observed following physiologic UV exposures (Friedmann and Gilchrest, 1987). These observations were recently confirmed and expanded by Pedeux *et al* (1998), who found a doubling of melanin content in both normal adult melanocytes and a pigmented human melanoma line after 7 d of pTpT treatment, accompanied by a proportional increase in ^{14}C-DOPA incorporation, confirming that enhanced melanin synthesis, as opposed to the accompanying cell growth retardation, accounted for the increase in melanin per cell (Pedeux *et al*, 1998).

To determine the mechanism of this response, paired cultures were again treated with UV or sham irradiation or with pTpT or diluent alone and harvested at intervals to determine the expression of tyrosinase, the rate-limiting enzyme in melanogenesis. Compared with sham-irradiated controls, UV-irradiated S91 cells increased tyrosinase mRNA and protein levels 2–3-fold as determined by northern and western blot analysis, respectively, after approximately 24 h, with persistence of the increased levels through at least 96 h. pTpT had very similar effects on tyrosinase expression (Eller *et al*, 1994). Because UV irradiation of S91 cells has also been shown to increase cell surface binding of α melanocyte stimulating hormone (αMSH) (Bolognia *et al*, 1989), a presumed mechanism of UV-induced tanning in these cells, in subsequent experiments ^{125}I-αMSH binding was also examined in S91 cells incubated in pTpT or diluent alone. An approximately 4-fold increase in MSH binding was observed within 72 h (Eller *et al*, 1996), again very comparable with the increase observed with physiologic UV exposures (Bolognia *et al*, 1989). Thus, at least two of the molecular mechanisms underlying UV-induced melanogenesis were found to be mimicked by pTpT.

In order to determine whether pTpT could produce a tanning reaction in intact skin, experiments were performed using a strain of guinea pigs developed for UV irradiation studies (Bolognia *et al*, 1989). Unlike most rodents, this animal has interfollicular epidermal melanocytes as well as hair follicle melanocytes, and the shaved skin is thus capable of a tanning reaction essentially identical to that observed in human skin. Guinea pigs were shaved and then chemically depilated to remove the normally thick coat, and areas on the flank with even skin color were selected as test sites. pTpT or vehicle alone (propylene glycol or dimethyl sulfoxide: propylene glycol 25:75) was applied once or twice daily for 1 or 2 wk (10 exposures total in most protocols). Little or no reaction was apparent during the period of applications, but beginning in the third week brown pigmentation gradually appeared in the pTpT-treated sites, usually becoming maximal at approximately day 21 (Eller *et al*, 1994). The pigmentation continued at maximal or near maximal levels for approximately 1 wk and then gradually faded to the original skin color in the absence of further treatment. Vehicle-treated sites showed either no increase in pigmentation or a very modest increase, far less than the pTpT-treated sites. Skin biopsies obtained at the time of maximal tanning response and subjected to Fontana-Masson staining to highlight melanin showed increased basilar and suprabasilar melanin in pTpT-treated sites, with distinct nuclear capping in many keratinocytes, identical to the histologic appearance of UV-induced tanning in this animal (Eller *et al*, 1994). There were no other consistent histologic changes compared with the vehicle-treated sites, which were in turn identical to untreated skin.

In both the *in vitro* and the *in vivo* experiments, pTpT was studied because it participates, as the substrate for thymine dimer formation, in the large majority of UV-induced photoproducts and hence would be the common denominator in most single-stranded DNA fragments excised during the course of DNA repair. To examine the specificity of pTpT in inducing melanogenesis, deoxyadenine dinucleotide (pdApdA) was used as a control, as this dinucleotide only rarely participates in DNA photoproduct formation under physiologic conditions (Gasparro and Fresco, 1986). Indeed, pdApdA had virtually no effect on either cultured pigment cells or on intact guinea pig skin (Eller *et al*, 1994). In later experiments further pursuing the question of specificity of the pTpT effects, however, DNA oligomers ranging in length from 5 to 9 bases and lacking adjacent thymidines were also tested in the S91 cell model. Interestingly, some but not all of the oligomers tested were active,[2] some several-fold more active than pTpT (**Fig 2**), suggesting that the thymidine dinucleotide is not required to stimulate melanogenesis in the cells. Work to date has not elucidated the oligomer features required for activity in this bioassay, such as base sequence, tertiary structure, or simply rate of metabolism into single nucleotides, known to be inactive (Pedeux *et al*, 1998; Eller and Gilchrest, unpublished data). Similarly, to date it is not known whether the oligomers exert

[1]Crowley DJ, Hanawalt PC. Induction of the SOS response increases the efficiency of global nucleotide excision repair of cyclobutane pyrimidine dimers, but not 6-4 photoproducts in UV-irradiated *Escherichia coli*. *Photochem Photobiol* 67:75, 1998 (abstr.)

[2]Eller MS, Gasparro FP, Amato PE, Gilchrest BA. Induction of melanogenesis by DNA oligonucleotides: effect of oligo size and sequence. *J Invest Dermatol* 110:474, 1998 (abstr.)

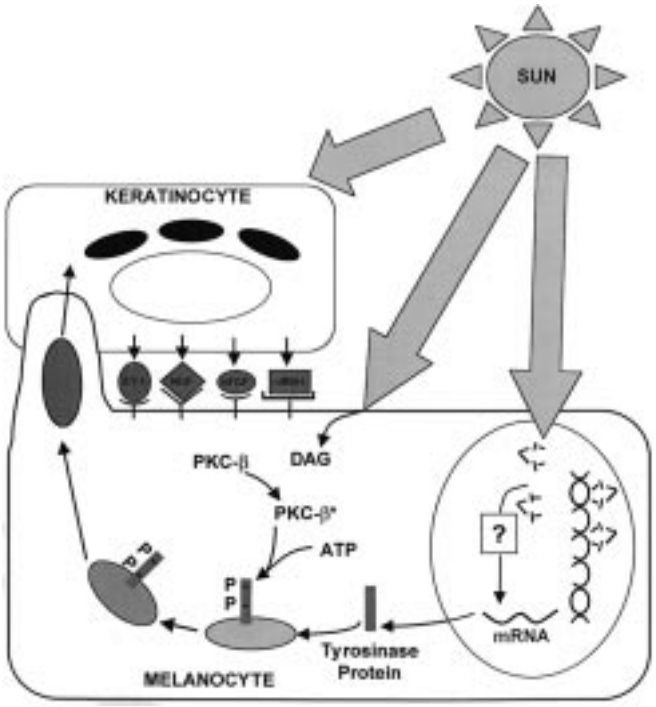

Figure 1. Direct and indirect effects of UV irradiation on melanogenesis. Within 24 h of a sufficient sun exposure, keratinocytes increase production and secretion of multiple paracrine factors, including endothelin-1 (ET-1), nerve growth factor (NGF), basic fibroblast factor (bFGF), and α-melanocyte stimulating hormone (αMSH). These and possibly other factors, released by cells in the dermis, bind their cognate receptors on the melanocyte surface, enhancing melanocyte survival, dendricity, and melanogenesis as well as stimulating cell division. UV photons also act directly on melanocyte membranes, cleaving diacylglycerol (DAG) that activates (*) protein kinase C (PKC), whose β isoform then activates the melanosomal protein tyrosinase by phosphylating its cytoplasmic domain. Increased melanogenic activity of the rate-limiting enzyme leads to more rapid and extensive melanin deposition in the melanosomes that are eventually transferred via dendritic projections to surrounding keratinocytes, where they are preferentially arrayed in photoprotective supranuclear caps. Simultaneously, UV photons interact with DNA in the nucleus, generating photoproducts, particularly thymine dimers, that are then excised over several days, releasing single-stranded DNA fragments. These DNA fragments then appear to stimulate transcription of several genes including tyrosinase, resulting over several days in gradual accumulation of tyrosinase protein, the rate-limiting enzyme in melanogenesis. Interactions among the postreceptor signal transduction molecules, DAG and other membrane lipid cleavage products, and nuclear transcription factors regulating expression of melanogenic gene products are highly likely but not yet elucidated.

their biologic affect in the cell nucleus, consistent with the initial hypothesis, or whether the underlying molecular events occur at another cellular location. It has been ascertained, however, that oligonucleotides concentrate in the nucleus (White *et al*., 1999)[3] agents capable only of damaging DNA, such as restriction enzymes and the DNA-damaging chemicals MMS and 4-NQO, are capable of stimulating melanogenesis via increased expression of tyrosinase and increased αMSH binding (Eller *et al*, 1996), consistent with the hypothesis that DNA fragments such as pTpT exert their effects by simulating excised UV-induced DNA photoproducts or other repair intermedia.

TANNING STIMULATED BY DNA FRAGMENTS IS PHOTOPROTECTIVE

Sun-induced tanning is known to be photoprotective, with a sun protection factor of approximately 3–5, depending on the individual's genetically determined ability to tan (Gilchrest *et al*, 1996). This photoprotective effect of increased epidermal melanin, measured for example by placing excised trypsin-split tanned epidermis over viable skin prior to UV irradiation, is in contrast to the virtual lack of photoprotection provided by, for example, topically applied dihydroxyacetone, which imparts a brown color to skin by binding to stratum corneum proteins, but which does not appreciably alter the minimal erythema dose (MED) of treated skin (reviewed in Pathak and Fitzpatrick, 1993).

In order to determine whether the pTpT-induced tan is photoprotective, guinea pigs were treated as described above; and after approximately 1 mo, when the treated sites were notably darker than the surrounding untreated skin, the shaved flanks of the animals were exposed to a previously determined 6 MED dose of UVB irradiation, known to be highly injurious. Twenty-four hours after the irradiation, at the height of the sunburn reaction, biopsies were obtained from the UV-irradiated untreated skin as well as from the equally irradiated pTpT-treated and clinically tanned skin (**Fig 3**). Biopsies were sectioned and stained with Fontana-Masson to indicate melanin content and with hematoxylin and eosin to reveal changes in the cellular architecture. As expected, Fontana-Masson-stained sections revealed a greater melanin content in pTpT-treated sites than in untreated sites. Also, the hematoxylin and eosin-stained sections of UV-irradiated untreated skin revealed extensive epidermal damage with necrosis of the upper layers and intraepidermal blister formation. In contrast, the pTpT-treated sites showed no histologic damage at all and were indistinguishable from nonirradiated sections except for the modest increase in basilar and suprabasilar melanin. Thus, the pTpT-induced tan was fully protective against at least a 6 MED UV dose.

DNA FRAGMENTS STIMULATE OTHER PHOTOPROTECTIVE RESPONSES

As noted above, in bacteria the UV-induced SOS response consists of transcriptional upregulation of genes involved in DNA repair and cell survival. Some recent data support the existence of a similar inducible response in mammalian cells (Protic *et al*, 1988; McKay and Rainbow, 1996; McKay *et al*, 1997). We therefore asked whether pTpT might also stimulate DNA repair capacity in human skin-derived cells.

If DNA repair capacity could be upregulated in human skin, it would be expected to have a clinically meaningful protective effect, in that impaired DNA repair capacity is known to be associated with the development of skin cancer. The most dramatic example of this relationship is the human disease xeroderma pigmentosum (XP), in which an inherited mutation in one of several genes whose protein products are required for normal DNA repair results in markedly compromised repair of UV-induced DNA photoproducts and a greater than 1000-fold increase in incidence of skin cancers, among other consequences of reduced DNA repair, such as progressive neurodegeneration (Kraemer *et al*, 1987). Occurence of skin cancers in otherwise normal individuals has also been linked to reduced DNA repair capacity. In particular, it has been shown (Wei *et al*, 1993) that otherwise normal individuals who experience at least one basal cell cancer before age 50 y have a statistically reduced DNA repair capacity compared with age matched controls with similar complexion and life-long sun exposure; and that among normal individuals without a history of skin cancer, there is an age-associated statistically significant decrease in DNA repair capacity between early and late adulthood (Wei *et al*, 1993) that is associated with an increased mutation frequency (Moriwaki *et al*, 1996) and an increased risk of skin cancer development in the latter decades of life (Scotto *et al*, 1983).

The data linking reduced DNA repair capacity with early onset skin cancer or advanced age were generated using a host cell reactivation assay in which a constitutively expressed bacterial chloramphenicol acetyl transferase (CAT) gene in an appropriate nonreplicating expression vector under a constitutive promotor, or the same CAT construct first UV-irradiated *in vitro*, is transfected into paired cultures of human cells. After 24 h the transfected cultures are harvested for a standard CAT assay. Cells transfected with the undamaged CAT construct give rise to abundant CAT protein and a correspondingly large amount of

[3]Eller MS, Hadshiew I, Gasparrow FP, Gilchrest BA. The effect of oligonucleotide size and 5-phosphate on stimulation of melanogenesis. *J Invest Dermatol* 112:541, 1999 (abstr.)

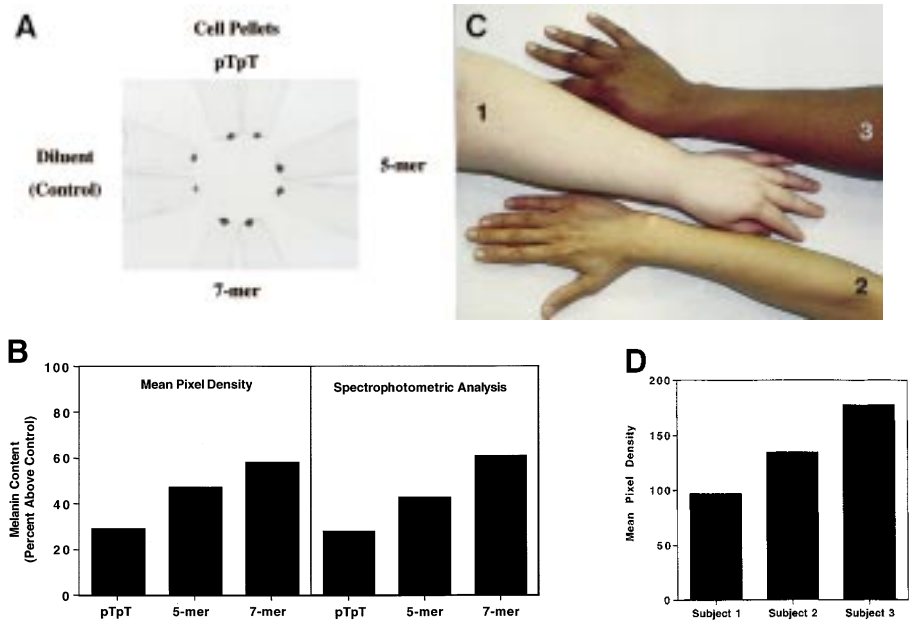

Figure 2. DNA fragments including pTpT stimulate pigmentation in human melanocytes that is clinically significant. Paired cultures of normal human melanocytes were maintained in duplicate for 6 d in vehicle alone or in 100 μM of pTpT, a 7 base oligonucleotide (5′AGTATGA3′) or a 5 base oligonucleotide (5′GTATG3′), generated by Dr Frank Gasparro, Department of Dermatology, Thomas Jefferson University. Cultures were then trypsinized and equal numbers of cells from each culture were pelleted. Note that because the pellet forms a meniscus in the bottom of the centrifuge tube, more darkly pigmented pellets appear larger, even though they are not, because the thin pellet edge remains visible. The same pellets were then analyzed for melanin content spectrophotometrically ($OD_{475\ nm}$) and the results expressed as a percentage of the diluent-supplemented control. With one exception, standard errors were less then 7% of the mean value. The photograph of the cell pellets was digitalized and analyzed by Dr. H. Randolph Byers, Department of Dermatology, Boston University School of Medicine, using densitometry image analysis software (IP Lab Spectrum, Scanalytics, Vienna, VA), and the results were again expressed as percentage of the diluent control. The spectrophotometric readings and the densitometry image analysis gave very comparable results but showed less than a doubling of melanin content, in intuitive disagreement with the visual appearance of the cell pellets. To explore the discrepancy between subjective investigator impression based on inspection of the pellets and the objective analyses, a photograph was obtained of the dorsal forearms of three laboratory members with skin types I, IV, and VI, representing the spectrum of human skin color. This photograph was then subjected to the same computerized image analysis as the cell pellets. As in that case, the darkest skin color was assigned a value less than twice that of the lightest skin color, confirming the investigators' suspicion that the human eye perceives greater differences in color than do these laboratory instruments. The data suggest that even modest differences in melanin content as determined spectrophotometrically or by computer-assisted image analysis may be highly clinically significant.

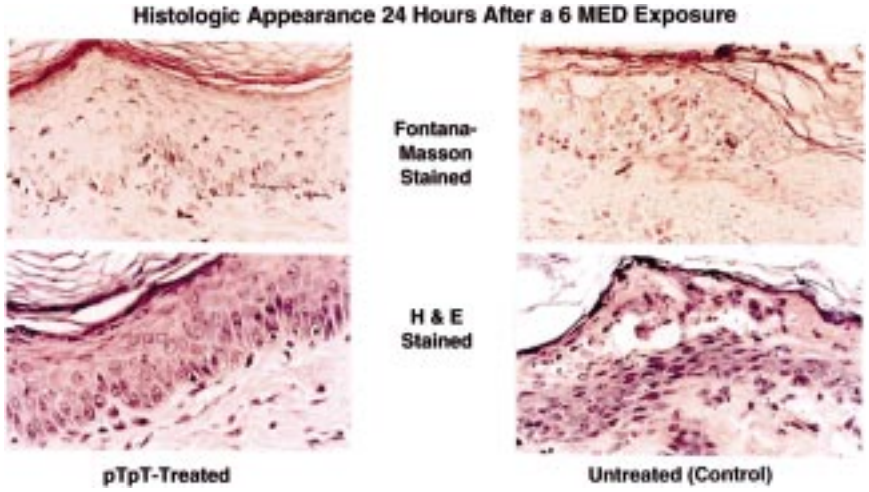

Figure 3. Dinucleotide-induced tanning is photoprotective. Tanning reaction was stimulated in guinea pig skin by topical application of pTpT 100 μM daily for 2 wk (10 d). One month after the first application, when the tan was at its peak, the animals were reshaved and the entire flank exposed to a previously determined 6 MED dose of UVB from a fluorescent source (FS 20 Sylvania bulbs). Twenty-four hours after the irradiation, at the expected height of the sunburn reaction, punch biopsies were obtained from the UV-irradiated previously untreated skin as well as from the equally irradiated pTpT-treated and tanned skin. Biopsies were formalin fixed, sectioned, and stained with Fontana-Masson to indicate melanin content (upper panels), and with hematoxylin and eosin (lower panels) to reveal changes in the cellular architecture. (Original magnification 40×).

acetylated chloramphenicol in the bioassay, while cultures transfected with the UV-irradiated CAT construct must first remove the DNA photoproducts before transcribing the CAT gene and hence give rise to less CAT activity. The better the host cell DNA repair capacity, the less the reduction in CAT activity.

To determine the effect of pTpT on DNA repair capacity in human skin-derived cells, paired cultures of epidermal keratinocytes or dermal fibroblasts were incubated in pTpT or diluent alone for 5 d and then transfected with either the undamaged CAT vector or the irradiated CAT vector, then harvested 24 h later for CAT activity. As expected on the basis of the previously published work (Wei *et al*, 1993), diluent-treated cultures transfected with the UV-damaged CAT construct gave rise to approximately 25% as much CAT activity as cultures transfected with the undamaged construct (Eller *et al*, 1997). In contrast, cultures pretreated for 5 d with pTpT and then transfected gave rise to more than 50% as much CAT activity as paired pTpT-treated cultures transfected with undamaged CAT construct. Thus, in the same assay system in which cancer-proneness is associated with an approximately 15% decrease in DNA repair capacity (Wei *et al*, 1993), pTpT treatment increased repair capacity by more than 100%.

In a second assay designed to assess the effect of pTpT on repair of UV-induced DNA photoproducts, paired dermal fibroblast cultures were incubated with pTpT or diluent alone and were then exposed to a damaging dose of UV irradiation. Immediately before, immediately after, and at intervals up to 24 h, DNA was isolated and slot blotted onto membranes that were then sequentially reacted with antibodies directed against cyclobutane pyrimidine dimers and (6–4) photoproducts, or hybridized with ^{32}P-labeled DNA fragments as a loading control. The blots were subjected to densitometric analysis and the rate of removal of photoproducts normalized against the loading control. In diluent-treated cells, as previously reported in the literature (Mitchell *et al*, 1990; Mitchell and Karentz, 1993; Eller *et al*, 1997), newborn control cultures removed approximately 50% of thymine dimers and nearly all (6–4) photoproducts within 24 h. In contrast, adult-derived cultures removed approximately 30% of thymine dimers and approximately 75% of (6–4) photoproducts after 24 h,[4] a statistically slower rate. Dinucleotide-treated adult donor cultures, however, removed both photoproducts at a statistically faster rate than controls, comparable with the rate observed in newborn cultures (Eller *et al*, 1997).

In order to determine whether pTpT treatment enhanced repair of thymidine dimers in intact UV-irradiated skin, paired sites on guinea pigs were treated with pTpT or diluent alone for 5 d, then exposed to a 0.5 MED dose of UV-B irradiation, using FS20 fluorescent bulbs. Biopsies were obtained from both sites after 6 h and tissue cross-sections were reacted with monoclonal antibodies specific for thymidine dimers. Skin pretreated with pTpT contained fewer dimers than did skin pretreated with diluent alone (**Fig 4**), and a computer assisted image analysis of the immunostained cross-sections revealed a dimer decrease of 44% $\pm$ 22% ($p < 0.04$, paired t test, $n = 4$ animals) compared with the immediate post-irradiation time point in pTpT-treated skin. Thymine dimer content in diluent-treated skin was essentially unchanged after 6 h.

MECHANISM OF PTPT-ENHANCED DNA REPAIR CAPACITY

Successful repair of UV-induced DNA damage relies in part on cell cycle arrest at the G1/S boundary, which theoretically increases the period of time available for nucleotide excision repair prior to DNA replication and mitosis (Murray, 1992). Also, at least in bacteria, there is upregulation of key DNA repair enzymes (Echols, 1991). In mammalian cells, the UV-induced cell cycle arrest is known to be mediated by upregulation and activation of the p53 tumor suppressor protein (Levine, 1997), also termed the "guardian of the genome" because of its central role in DNA repair and apoptotic cell loss following genotoxic cellular injury (Marx, 1994).

pTpT-treated cells were observed to reversibly decrease their proliferative rate and to upregulate the message for the p53-regulated p21 protein known to mediate cell cycle arrest, consistent with UV-mimetic p53 activation (Eller *et al*, 1997). pTpT-treated cells also exhibited nuclear translocation of the p53 protein, a recognized indication of p53 activation, as well as enhanced affinity of nuclear p53 protein for its DNA consensus sequence, strong direct evidence of p53 activation by pTpT (Eller *et al*, 1997). pTpT treatment of human cells was subsequently also shown to increase read-out of a human growth hormone transgene under control of a promoter containing the p53 consensus element (Maeda *et al*, 1999). Finally, pTpT induced transient growth arrest and p21 upregulation in the p53-null H1299 cell line only after transfection of the cell line with a wild-type p53 expression vector (Eller *et al*, 1997). In combination, these data unequivocally demonstrate that pTpT acts at least in part through activation of p53 and constitute a further parallel between pTpT-mediated and UV-mediated cellular effects. Although p53 can mediate apoptosis in severely damaged cells, no apoptosis of pTpT-treated cells has been detected morphologically by the DNA end-labeling TUNEL assay, by FAC Scan analysis for nuclear fragmentation, or by determination of colony-forming efficiency of pTpT-treated cells (Eller *et al*, 1997 and unpublished data; Pedeux *et al*, 1998).

In recent studies, our group has sought to identify the specific DNA

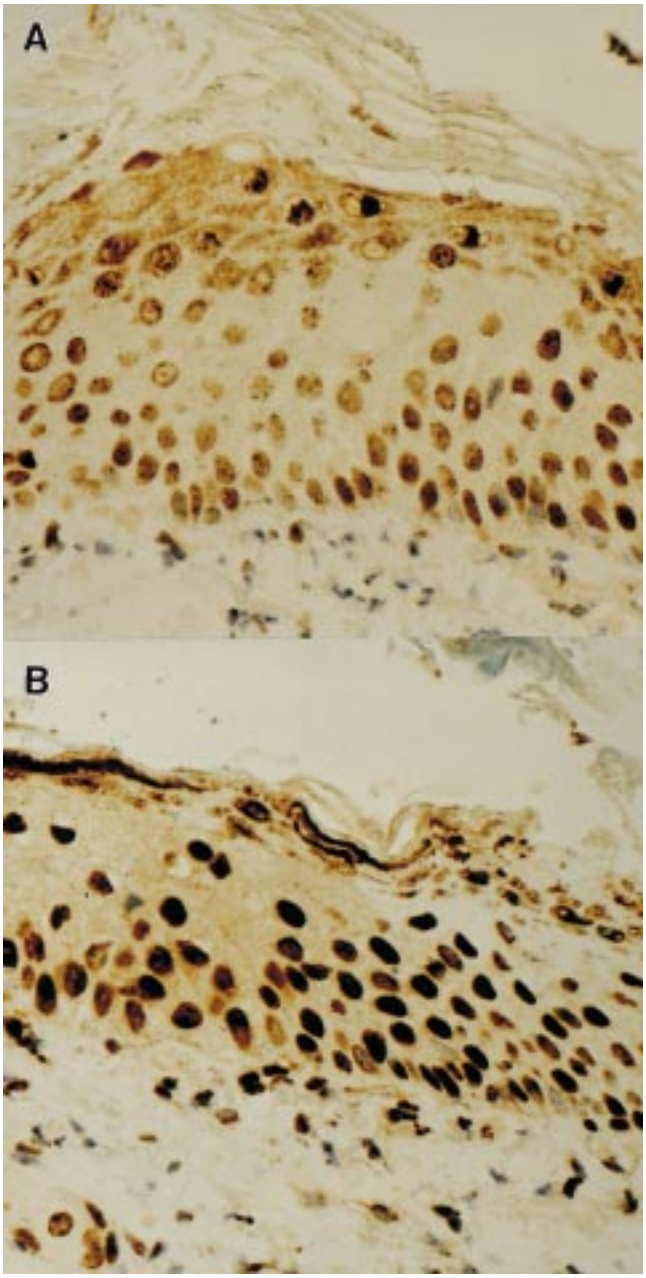

Figure 4. pTpT 100 μM or diluent alone was applied daily for 5 d to shaved depilated guinea pig skin. On day 6, the pTpT-treated area, which had not yet visibly darkened, and the adjacent area of diluent-treated skin were exposed to a 0.5 MED dose of UVB (Sylvania FS 20 bulbs). Six hours later punch biopsies were obtained from the UV-exposed pTpT-treated and diluent-treated skin. Frozen sections were then provided to Professor Christopher S. Potten, University of Manchester, U.K., reacted with monochronal antibodies specific for thymine dimers and the immunostained cross-sections subjected to computerized image analysis (unpublished data, Potten and Chadwick, CRC Epithelial Biology Department, Paterson Institute, Manchester, U.K. and Eller, Goukassian and Gilchrest, Department of Dermatology, Boston University School of Medicine). Biopsies obtained immediately post-irradiation showed equal dimer antibody binding (data not shown), as expected, but after 6 h there was a noticeable reduction in the pTpT-treated skin (*A*) compared with the diluent-treated (control) skin (*B*). Image analysis revealed a statistically significant ($p < 0.04$) reduction in dimers in the pTpT-treated skin. Biopsies from one of four animals are shown. (Original magnification 40×).

[4]Gad F, Yaar M, Eller M, Gilchrest BA. The DNA repair capacity of human fibroblasts declines with donor age. *J Invest Dermatol* 110:690, 1998 (abstr.)

repair enzymes and cell cycle regulatory proteins upregulated by pTpT. To date, several proteins recognized to be p53 regulated, such as p21, proliferating cell nuclear antigen (PCNA), and GADD45, as well as certain other proteins not recognized to be p53 regulated, such as the XPA correcting protein known to be mutated in complementation XP group A patients, were all found to be upregulated at the mRNA and/or protein levels by pTpT treatment (Eller *et al*, 1997; Goukassian *et al*, 1999). Moreover, while these proteins are also upregulated by UV irradiation, cells pretreated with pTpT for 5 d prior to UV irradiation have 2–5-fold higher increases than do diluent-pretreated cells (Goukassian *et al*, 1999), consistent with the previously observed increase in DNA repair rate following UV irradiation (Eller *et al*, 1997).

SUMMARY AND CONCLUSIONS

Mammalian skin responds to UV irradiation by increased production of the pigment melanin in melanocytes, with subsequent distribution to surrounding keratinocytes in a manner shown to be photoprotective. Also, recent data indicate that mammalian cells, like bacterial cells, have a UV-inducible DNA repair capacity that further protects the tissue from subsequent UV exposure. The combined effect of UV-induced melanogenesis (tanning) and enhanced DNA repair capacity, as well as possibly other as yet poorly elucidated inducible responses, is to render the skin far more resistant to subsequent UV injury. Such responses can reasonably be presumed important in protecting skin from acute and chronic UV damage, including development of skin cancer. These SOS-like responses, normally induced by UV irradiation or other DNA injury, appear to be mimicked in the absence of DNA damage by thymidine dinucleotides and other single-stranded DNA fragments, suggesting that the SOS response is normally mediated at least in part by excised DNA fragments. This observation suggests the possibility of long-term minimization of cutaneous DNA damage by topical treatment with small DNA fragments. This novel approach to photoprotection would permit the acquisition of a cosmetically pleasing photoprotective tan while conferring increased resistence to future UV damage.

These data further establish that pTpT upregulates DNA repair capacity at least in part by upregulation of the mRNA and protein levels of the responsible repair proteins; and that this response is large, constituting at least half of the cell's maximal DNA repair capacity. In mammalian cells, beyond the evolutionarily conserved increase in DNA repair capacity, the SOS response appears to include enhanced melanogenesis by epidermal melanocytes, due in part to upregulation of the rate-limiting enzyme tyrosinase, which contributes independently to reducing DNA damage through absorbing UV photons and UV-induced free radicals.

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